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Capillary zone electrophoresis in mixed aqueous–organic media: effect of organic solvents on actual ionic mobilities, acidity constants and separation selectivity of substituted aromatic acids II. Ethanol

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Abstract

The influence of the composition of mixed aqueous–ethanolic solvents on the actual mobilities and the apparent ionization constants of 26 aromatic acids with methyl, nitro, chloro and hydroxy substitution was investigated, with concentrations ranging up to 80% (v/v) ethanol. Mobilities were determined by capillary zone electrophoresis (phosphate buffer, pH 7 in water, ionic strength 20 mmol/l), the mixed-mode, apparent pK_a values by potentiometric titration. Increase of the pK_a values by about 1.8–2.6 units was found for 80% EtOH, about 0.5 units higher compared to methanolic solvents with the same composition. A drastic decrease of the actual mobilities with increasing ethanol concentration was found; this decrease is less pronounced considering the change of the viscosity of the mixed solvent. Taking into account the product of actual mobility with viscosity of the solvent (the Walden product), only considerably small changes of this parameter with solvent composition are observed on the other hand. Based on these changes, and also based on the selectivity coefficients (the ratios of the actual mobility of benzoate to that of the respective ionic solute) a classification of the solutes into two distinct groups is found: one class consists of all non-hydroxy-substituted solutes; the second contains all analytes with OH substituents. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

In capillary electrophoresis the total mobility, $\mu_{\text{tot},i}$, of solute, i , is the property decisive for migration. It consists of the effective mobility, $\mu_{\text{eff},i}$, and the mobility of the electroosmotic flow (EOF),

μ_{eof} . For weak acids or bases, respectively, as solutes with ionization constants K_a , and for the normally used separation capillaries (made from silica material) these mobilities depend on the pH of the separation buffer, its ionic strength and the temperature. Other processes except protolysis that might influence the concentration of the free ions, e.g. complexation reactions, also influence the mobility, because they determine the degree of ionization, α , and thus the total mobility expressible by

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$$\mu_{\text{tot},i} = \mu_{\text{eff},i} + \mu_{\text{eof}} = \mu_{\text{act},i}\alpha + \mu_{\text{eof}} \quad (1)$$

with $\mu_{\text{act},i}$ being the mobility of the fully charged solute at the ionic strength of the solution.

In case of protolysis, e.g. by ionization of mono-valent acids or bases, respectively, the total mobility is expressed by

$$\mu_{\text{tot},i} = \frac{\mu_{\text{act},i}}{1 \pm 10^{\text{p}K_{\text{a}} - \text{pH}}} + \mu_{\text{eof}} \quad (2)$$

From Eq. (2) it can be deduced how the total mobility can be influenced:

(i) the mobility of the EOF can be changed unspecifically by variation of the pH and the ionic strength. An additional possibility is to change the solvent composition. Besides the most popular aqueous buffer solutions either mixed aqueous–organic or nonaqueous solvents can be applied. The increment in mobility caused by the EOF is obviously the same for all separands. However, it should be pointed out that the EOF in fact may have a severe impact on efficiency, selectivity and resolution. It can be demonstrated that under conditions with the EOF directed towards the cathode, the resolution of cationic separands is always reduced compared to the situation without an EOF. Anionic separands, however, can exhibit better or lower resolution in presence of an EOF compared to the EOF-free system, depending on the magnitude of the electrophoretic and electroosmotic mobility [1–3]. Further,

(ii) the actual mobility can be influenced either by the ionic strength or, more powerfully, by the variation of the solvent composition; and finally

(iii) the $\text{p}K_{\text{a}}$ values of the solutes can be changed by the ionic strength and, again, by varying the solvent composition.

It comes out that the composition of the solvent has the potential to influence the mobility of analytes in all important aspects [4–6]. For practical reasons the number of organic cosolvents in mixed aqueous–organic media in capillary zone electrophoresis (CZE) seems limited. For our investigation the lower alcohols and acetonitrile were selected, because these solvents are fully miscible with water, show low absorbance in the common UV range, have considerably good solubility properties for ionic or ionizable analytes or buffer electrolytes, and are favorable for manipulation and toxic reasons.

In a series of investigations the influence of these solvents on the actual mobility and the apparent $\text{p}K_{\text{a}}$ values of substituted aromatic acids was investigated. It was the intention of this work to clearly separate the influence of the solvent composition on these physico–chemical parameters rather than to study a certain separation problem by adding organic solvent to the buffer. By the latter strategy, which is followed in most cases in the literature (cf. e.g. Ref. [5]), normally no distinction is made concerning the mutual influence of separation selectivity (either by changing the actual mobility, or the $\text{p}K_{\text{a}}$, or both) and efficiency (including the influence of the EOF on peak broadening due to the changed residence time in the separation capillary). Therefore, in the present investigation the conditions are chosen such that the effect of the organic solvent on the actual mobility and the apparent $\text{p}K_{\text{a}}$ becomes clear in a general manner. The change of the electroosmotic mobility by variation of the solvent composition was already the topic of previous papers [7,8], and is not of interest here. In this paper the influence of ethanol (EtOH) is discussed, which follows a previous work dealing with methanolic solvents [4], and is in a series that will be completed with 1-propanol and acetonitrile as cosolvents.

2. Experimental

2.1. Instrumentation

2.1.1. Electrophoresis

The CE instrument used was a programmable P/ACE 2100 CE unit (Beckman Instruments, Fullerton, CA, USA) with a variable-wavelength UV detector worked at 214 nm. Untreated fused-silica capillaries (Polymicro Technologies, Bloomfield, NJ, USA) were applied in thermostatted cartridges at 25.0°C for all measurements. Capillaries were conditioned daily by rinsing for 5 min with 0.1 M NaOH, 5 min with bidistilled water and finally with the running buffer for additional 5 min. Rinsing in between two runs for 1 min with bidistilled water and for 2 min with background electrolyte (BGE) was performed in the high pressure mode (140 kPa). The capillary was stored empty overnight.

A constant voltage of 10.0 kV with a linear voltage

ramp-up set at 0.17 min was used during all experiments. Taking into account the ramp into the calculation exhibited a significant influence on the resulting mobility values. Measurements were carried out in duplicate, typically leading to data varying within 1%. For data collection and processing Gold Software 3.0 was employed.

10 mmol/l phosphate buffer, made from 5 mmol/l Na_2HPO_4 and 5 mmol/l NaH_2PO_4 , was used as the BGE throughout all measurements (the resulting ionic strength was 20 mmol/l).

The samples had a concentration of 1×10^{-4} mol/l. Injections were performed in the low-pressure mode (3.5 kPa) for 1 s.

The electrophoretic runs without pressure mobilization steps were carried out in a 50- μm I.D. \times 375- μm O.D. capillary with a total length of 26.4 cm and a length to the detector window of 19.5 cm. Experiments including mobilization by pressure – necessary only for the determination of the actual mobilities in 60% EtOH – required a capillary (75 μm I.D. \times 375 μm O.D.) with total length of 46.4 cm and effective length of 39.6 cm. All runs containing steps with a combination of an electrophoretic and a pressurized mode were carried out according to the method described by Williams and Vigh [9]: as a first step, low pressure (3.5 kPa) was applied for 2 min in order to shift the injected sample and dimethyl sulphoxide (DMSO) as EOF marker into the capillary. Then, a constant voltage of 10.0 kV was applied for 6 min. Finally, DMSO was introduced by injection for 0.02 min and all sample zones were mobilized by pressure (3.5 kPa) through the detection window.

Currents during runs were 11.7 μA at 0%, 7.6 μA at 20%, 5.2 μA at 40% and 4.0 μA in the combined pressure–voltage mode at 60% (v/v) EtOH.

2.1.2. Potentiometric determination of the apparent pK_a

For the determination of the apparent pK_a values of the selected acids ($c = 10$ mmol/l) in the mixed solvents potentiometric titration was applied with 100 mmol/l NaOH as titrator. For simplicity the titrator was a pure aqueous solution. The final composition of the solvent at $\text{pH} = pK_a$ was determined by calculation.

A Mettler titration apparatus (model DL 67,

Mettler Toledo, Schwerzenbach, Switzerland) equipped with a glass–calomel electrode (DG 101-SC, Mettler Toledo) filled with aqueous KCl, 3 mol/l, saturated with AgCl was used.

2.2. Chemicals

All aromatic acids used in this work were purchased from EGA-Chemie (Steinheim, Germany) with the highest grade of purity available. Na_2HPO_4 , NaH_2PO_4 and ethanol – all of analytical grade – were obtained from E. Merck (Darmstadt, Germany). Apparent pH values of the aqueous–organic running buffers used, determined by a glass electrode, were 7.02 at 0%, 7.47 at 20%, 7.95 at 40% and 8.50 at 60% (v/v) EtOH, indicating the change of the pK_a value of the phosphate buffer by the solvent.

Water used as solvent or as cosolvent was distilled twice before use from a quartz apparatus.

3. Results and discussion

3.1. Influence of ethanol on the apparent pK_a values of neutral acids

The change of the apparent pK_a values with the variation in composition of the solvent can be seen from Table 1 for the series of monosubstituted benzoic acids, all derivatized in the β -position to the carboxylate group on the aromatic ring. Note that the mixed-mode, apparent values are under discussion, not the thermodynamic pK_a values. They increase with increasing ethanol concentration. An even better insight on the increasing effect is given in Fig. 1, where the difference of the pK_a values in the respective mixed solvent to that in pure water is depicted. It is seen that the pK_a values increase nonlinearly by about 1.8–2.6 pK units at about 80% (v/v) ethanol, values nearly 0.5 pK units higher compared to methanol at the same concentration.

The measured data for both organic cosolvents can be fitted by third-order polynomials, according to

$$\Delta pK_a = pK_a^{\text{mix}} - pK_a^{\text{w}} = a_0 + a_1c + a_2c^2 + a_3c^3 \quad (3)$$

with the suffix mix representing the mixed aqueous–organic solvent, and w water; a is the coefficient of

Table 1

Apparent pK_a values of benzoic, 3-hydroxy-(3-OH), 3-methyl-(3-Me), 3-nitro-(3-NO₂) and 3-chloro-(3-Cl) benzoic acids in water and in mixed aqueous-ethanolic solutions

% (v/v) EtOH	Apparent pK_a value				
	Benzoic (A)	3-OH (C)	3-Me (L)	3-NO ₂ (S)	3-Cl (Y)
0	4.12	4.03	4.24	3.46	3.82
19.5	4.52	4.47	4.61	3.74	4.20
39.1	5.18	5.14	5.33	4.22	4.74
58.6	5.85	5.82	5.95	4.69	5.31
78.2	6.52	6.62	6.69	5.30	5.96

$I=5$ mmol/l, $T=25^\circ\text{C}$. Symbols in parentheses as in Tables 2 and 3. Precision of the potentiometric determination, given by the range of measurements in triplicate, is about 0.02 pK units.

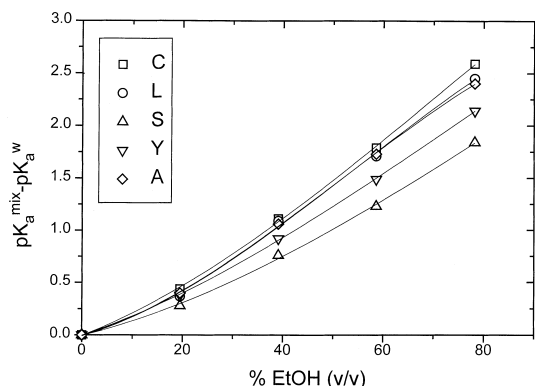


Fig. 1. Effect of the composition of the mixed aqueous–ethanolic solvent (mix) on the apparent, mixed-mode pK_a values of five *meta*-substituted benzoic acids, compared to water (w). The pK_a values were determined by potentiometric titration. Ionic strength, $I=5$ mmol/l. Symbols of the acids according to Table 1.

regression and c the concentration of ethanol in the mixed solvent in percent (v/v).

The resulting coefficients are given in Table 2, together with the values derived for mixed aqueous–

methanolic solvents [4]. It can be seen that for both cosolvents the intercepts a_0 (the regression coefficients) are not significantly different from zero, as proved by a t -test: the standard deviation of the estimation of a_0 is about one magnitude larger than the values of a_0 themselves. This means that in all solvent systems the pK_a values in water calculated by curve fitting obey the measured values.

The contrary is the case for the standard deviation obtained by calculation of a_1 , the linear part of the regression function: the standard deviation is here one magnitude smaller than a_1 . The value of a_1 for the ethanolic solvents is several times larger than that in methanol, which means that the linear part determines the steeper increase in ethanol compared to methanol. Considering the other two coefficients, a_2 and a_3 , reflecting the dependence of the curve on the second and the third power, respectively, on the organic cosolvent concentration, it is remarkable that these two latter coefficients do not differ in most cases for both cosolvents.

However, although the standard deviations of the estimation of these two coefficients are considerably

Table 2

Comparison of the parameters of polynomial regression, a , of the apparent pK_a value vs. percentage, c (in %, v/v), organic cosolvent methanol or ethanol, respectively, for the acids given in Table 1 and Fig. 1

Acid	a_0 (10^{-3}) (S.D.)		a_1 (10^{-2}) (S.D.)		a_2 (10^{-4}) (S.D.)		a_3 (10^{-6}) (S.D.)		r	
	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH
A	0.72 (6)	-3.3 (27)	0.66 (0.08)	1.4 (0.4)	4.1 (0.3)	4.4 (1.1)	-2.2 (0.2)	-2.91 (1.0)	0.99999	0.99989
C	1.0 (8)	-4.5 (38)	0.92 (0.12)	1.9 (0.5)	3.7 (0.4)	2.8 (1.6)	-2.0 (0.3)	-1.29 (1.3)	0.99999	0.99983
L	-0.43 (4)	-9.4 (78)	0.40 (0.05)	1.4 (1.0)	4.9 (0.1)	4.2 (3.3)	-2.9 (0.1)	-2.58 (2.8)	0.99999	0.99922
S	-3.5 (29)	-5.0 (42)	0.19 (0.37)	1.2 (0.5)	4.9 (1.2)	2.0 (1.8)	-2.8 (1.0)	-0.68 (1.5)	0.99973	0.99959
Y	2.0 (17)	-2.4 (20)	0.51 (0.21)	1.6 (0.3)	3.2 (0.7)	2.1 (0.8)	-1.5 (0.6)	-0.90 (0.7)	0.99992	0.99993

Polynomial regression was made according to Eq. (3). S.D., standard deviation; r , coefficient of correlation.

large, a much lower correlation is found if the third power fit is not taken into account to fit the curve to the data: the correlation coefficients (given in Table 2 for the regression according to Eq. (3)) have one or even two 9's less in these cases. For an appropriate fitting a third-power polynomial regression is in fact necessary.

Taking into account the similarity of the regression coefficients of the second and the third power, it follows that the significant difference of the shape of the pK_a vs. % (v/v) cosolvent curves is determined mainly by the linear part of the regression equation (Eq. (3)). If this part is not taken into account, the resulting curves of the particular acids in methanolic and ethanolic solvents of the same composition would in fact not differ. As the linear part of Eq. (3) plays a main role only at small concentrations of the organic solvent, it seems that even a low concentration of this solvent exhibits a decisive impact on the differences of the pK_a value changes.

The variation of the pK_a values with changing solvent composition can be explained by the concept of the transfer activity coefficient, ${}_m\gamma_i$ [10,11], of species, i , as discussed in previous papers [12,13]. It should be pointed out that Born's approach to explain the changes in pK_a values, which is based only on the electrostatic effects on the ion equilibria in the respective solvents leads to unsatisfactory results in most cases. This approach is explicitly not dealing with solvation effects, whereby it is especially solvation which plays the decisive role when considering ions in protic or apolar diprotic solvents. Therefore the more adequate approach is that based on the medium effect (the transfer activity coefficient, or its logarithm, respectively).

For a neutral acid HA (forming the ions H^+ and A^- after dissociation) this concept leads to an expression for the change of the pK_a value when the acid is transferred from water to the mixed solvent, given by

$$\begin{aligned} \Delta pK_a &= pK_a^{\text{mix}} - pK_a^{\text{w}} = \log \frac{{}_m\gamma_{H^+} + {}_m\gamma_{A^-}}{{}_m\gamma_{HA}} \\ &= \log {}_m\gamma_{H^+} + \log {}_m\gamma_{A^-} - \log {}_m\gamma_{HA} \end{aligned} \quad (4)$$

It should be pointed out that the free energy of transfer, $\log {}_m\gamma_i$, has negative values when the particle is better stabilized in the organic solvent than

in water, and has positive values when it is more stable in water than in the organic or mixed aqueous–organic solvent.

The medium effect on the proton reflects the basicity of the solvent, compared to water. As the values of the medium effect of the single species are obtained by approximation methods, they may differ depending on the type of approximation. However, positive values for the medium effect on the proton in ethanolic solvents is reported in most investigations, indicating that ethanol is less basic than water. The dependence of $\log {}_m\gamma_{H^+}$ on the ethanol concentration, taken from results published by Izmailov [14–16] and Grunwald and co-workers [17–19], respectively, are shown in Fig. 2. The values differ only slightly, and show that the basicity of the mixed aqueous–ethanolic solvent decreases slightly with increasing ethanol concentration, whereas over 70% ethanol the increase becomes by far steeper.

If the contribution of the medium effect on the proton is subtracted from ΔpK_a , the effects of the contribution of the solvation of the anion A^- , and that of the neutral acid HA remains. The resulting curves are depicted in Fig. 2 for 3-hydroxybenzoic acid; they show the dependence of the medium effect on the anion, A^- , and that on the neutral species, HA.

The resulting differences

$$\Delta pK_a - \log {}_m\gamma_{H^+} = \log {}_m\gamma_{A^-} - \log {}_m\gamma_{HA} \quad (5)$$

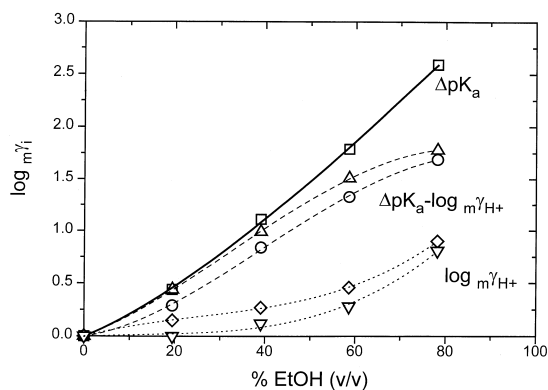


Fig. 2. Total medium effect on 3-hydroxybenzoic acid, $\log {}_m\gamma_i = \Delta pK_a$, the medium effect on the proton, $\log {}_m\gamma_{H^+}$, and the difference according to Eq. (5), $\log ({}_m\gamma_{A^-} / {}_m\gamma_{HA})$, on the solvent composition. Data for $\log {}_m\gamma_{H^+}$ were taken from Refs. [14–16] (\diamond) and Refs. [17–19] (∇). ΔpK_a values according to Table 1 and Fig. 1.

increase with increasing ethanol content, and give a value of nearly 2 at 80% EtOH compared to water.

For a closer inspection of the resulting effect of the solvent on the pK_a value the source of the medium effect on the neutral species HA is related to the stabilization of this particle in both solvents. It is reflected by the solubility of this species in both solvents, expressed by

$$\log {}_m\gamma_{HA} = \log \frac{{}^wK_{s0}}{\text{mix}K_{s0}} \quad (6)$$

where ${}^wK_{s0}$ and $\text{mix}K_{s0}$ are the solubility products of solute HA in water and the mixed solvent, respectively [20].

As the free organic acids under consideration are better soluble in the mixed solvent than in pure water, a (slight) negative value of the medium effect on HA results. This means that this effect leads to a (slight) increase of the ΔpK_a values (see Eq. (4)). However, this effect is not large enough to account for the remaining increase of the pK_a value by nearly 2 units (see Eq. (5)). The more decisive influence originates from the poor anion solvation ability of ethanol compared to water: negatively charged ions are by far less stabilized by solvation in ethanol than in water. This worse anion solvation ability characterizes organic solvents: in most cases the pK_a values increase upon addition of organic cosolvent, almost independent of its basicity.

3.2. Mobilities

The actual mobilities of the 26 solutes in the mixed ethanolic–aqueous solutions are given in Table 3. The ethanol concentration was limited in the present investigation to 60% (v/v), because at higher ethanol contents the buffer solutions used for the CZE measurements of the mobilities became turbid due to the reduced solubility of the buffering electrolyte in the solvent, possibly leading to precipitation inside the capillary during the measurement. Other, better soluble electrolytes than the phosphate buffer used here were not applied to extend the concentration range of ethanol, because it was our intention to maintain the experimental conditions in the entire series of investigations as consistent as possible.

Table 3

Actual mobilities, $\mu_{act,i}$, of the anionic solutes in different aqueous–ethanolic solvents measured by CZE

Symbol	Name	$\mu_{act,i}$, % (v/v) EtOH			
		0	20	40	60
A	Benzoic	33.27	20.53	15.00	13.47
B	2-OH	36.29	21.01	14.96	13.80
C	3-OH	31.17	17.62	12.13	10.38
D	4-OH	31.08	17.25	11.90	9.90
E	2,3-diOH	32.62	18.17	13.09	12.26
F	2,4-diOH	32.53	17.42	11.99	11.08
G	3,4-diOH	29.68	16.05	11.18	9.59
H	3,5-diOH	28.97	15.80	10.79	9.49
I	2,4,6-triOH	34.00	17.43	12.01	10.65
J	3,4,5-triOH	27.69	15.08	10.39	9.34
K	2-Me	31.59	19.22	14.39	13.12
L	3-Me	31.53	18.88	13.88	12.47
M	4-Me	31.51	18.72	12.90	12.20
N	2,4-diMe	29.21	17.00	12.66	11.79
O	2,5-diMe	29.36	17.47	13.19	12.09
P	3,4-diMe	29.38	17.37	12.76	11.44
Q	3,5-diMe	28.94	17.37	12.70	11.65
R	2-NO ₂	32.78	19.77	15.00	13.50
S	3-NO ₂	32.27	19.27	13.84	12.50
T	4-NO ₂	32.65	19.29	14.05	12.78
U	3,4-diNO ₂	31.03	18.32	12.88	11.80
V	3,5-diNO ₂	30.63	18.36	13.51	12.52
W	2,4,6-triNO ₂	28.42	17.06	12.77	11.68
X	2-Cl	32.34	19.56	14.75	12.82
Y	3-Cl	32.63	19.31	13.96	12.49
Z	4-Cl	32.25	19.00	13.28	12.30

Ionic strength, 20 mmol/l; temperature, 25.0°C. Mobilities are given in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. The data for 0% EtOH are the same as in Ref. [4].

The actual mobilities of all solutes decrease with increasing ethanol concentration, and this reduction is more pronounced with EtOH compared to MeOH. At 60% EtOH the values of the actual mobilities are decreased to nearly one-third of those in pure aqueous buffer solution.

The correlation with the mobility data in pure aqueous solutions is lower with ethanol than with methanol as organic cosolvent, as can be seen from Fig. 3. The coefficients of linear correlation, r , are lower at low ethanol concentration, compared to aqueous–methanolic solvent with the same composition, e.g. at 20 and 40%. However, at 60% organic cosolvent, nearly no difference is found in the correlation of the respective mobilities with those in pure aqueous buffers: the coefficient of linear correlation is 0.59 (60% EtOH) and 0.63 (60%

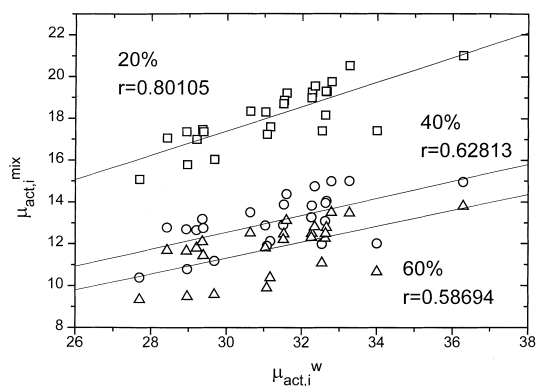


Fig. 3. Correlation of the actual mobilities, $\mu_{act,i}$, of the solutes (Table 3) in the mixed aqueous–ethanolic (mix) solvents to those in pure aqueous buffers (w). (□) 20% EtOH; (○) 40% EtOH; (△) 60% EtOH.

MeOH), indicating in both cases the lack of accordance when comparing the data in the mixed solvent with those in pure water.

A better correlation is found when the mixed solvents with the same concentration of the lower alcohols are compared, as shown in Fig. 4: all coefficients of linear correlation, r , are better than 0.9. The lowest value for r is observed at the lowest concentration of the organic cosolvents, at 20%.

The decrease in actual mobilities (Table 3) goes hand in hand with an increase in viscosity of the mixed solvent (cf. e.g. Ref. [8]). This is in fact what

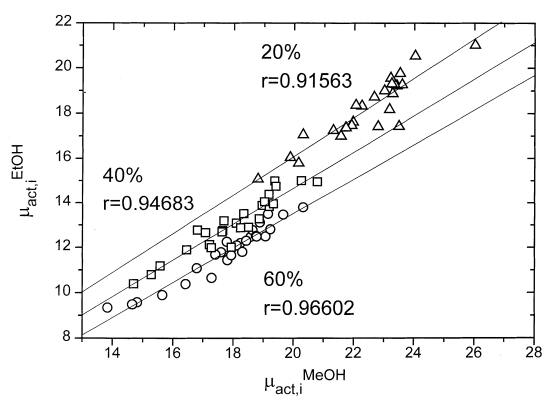


Fig. 4. Correlation of the actual mobilities, $\mu_{act,i}$, of the anions (Table 3) in aqueous–ethanolic buffers (EtOH) to the values in aqueous–methanolic buffers (MeOH) of the same concentration of the organic cosolvent (Ref. [4]). (△) 20% alcohol; (□) 40% alcohol; (○) 60% alcohol.

Walden's rule predicts, namely that the product of mobility and viscosity is constant (note that this rule is postulated for infinite dilution). The dependence of the product $\mu_{act,i}\eta$ is shown in Fig. 5 (the values of the viscosity, η , were 0.89 cP for water, 1.62 cP for 20%, 2.25 cP for 40% and 2.32 cP for 60% EtOH). Indeed this product changes much less with solvent composition than the actual mobility itself. Whereas the latter is reduced by a factor of up to 3, the former changes only within 20%.

For methanolic solvents the anions under consideration were found to form two groups with regard to the change of the Walden product. One group contained all anionic derivatives with chloro, methyl or nitro substituents, the other group all anions with OH substituents. The same grouping is found here for mixed aqueous–ethanolic solvents. The relative

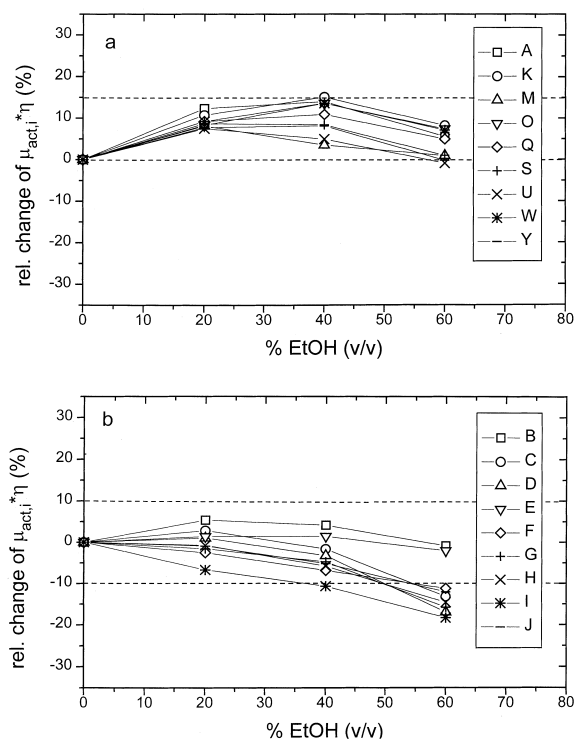


Fig. 5. Plots of relative changes of Walden products for the two different groups of anions in the various solvents. Data are shown for (a) the non-hydroxy-substituted and (b) the hydroxy-substituted anions. The symbols of the anions are according to Table 3. The relative changes are expressed by $100[(\mu_{act,i}\eta)^{mix} - (\mu_{act,i}\eta)^w]/(\mu_{act,i}\eta)^w$ (%); suffixes mix and w stand for mixed aqueous–organic solvent or water, respectively.

change of the products $\mu_{\text{act},i}\eta$ in the former group increase with increasing ethanol concentration, and reaches values of about 15% higher at 40% EtOH compared to pure water (Fig. 5a). At higher concentration the relative values of these products decrease slightly, and reach values comparable with water.

In contrast to this group of compounds, the second class containing almost all hydroxy-substituted anions shows a smaller increase, or even a decrease of the relative values of the Walden products (Fig. 5b). In fact the majority of these anions exhibit relative changes of the products at 60% EtOH that are significantly smaller than those in water. This means that the mobility of these anions is more strongly affected by increasing ethanol concentration than simply by viscosity.

Concluding, it is pointed out that the behavior of the anions in the ethanolic solvent is similar to that in the methanolic one of the same concentration. Whereby one can find a small deviation for the non-OH-substituted anion (the change of the relative values of $\mu_{\text{act},i}\eta$ ranges between -5 and $+10\%$ for methanolic solvents, and between 0 and $+15\%$ for the ethanolic systems) the hydroxy-substituted anions exhibit the same general trend in both solvent systems. This does not mean that in the particular solvents no specific effects on the products of a certain pair of solutes take place.

3.3. Selectivity

Changes in separation selectivity discussed are based on the effect of the solvent composition on the selectivity coefficient, the ratio of the actual mobilities of unsubstituted benzoic acid (A in the Tables) to separand, i , by

$$r_{A,i} = \frac{\mu_{\text{act},A}}{\mu_{\text{act},i}} \quad (7)$$

It is obvious that the parameter which is relevant for the separation of analytes is given by the difference of the mobilities of a pair of consecutively migrating ions, i and j , related to their mean mobility, $\bar{\mu}$, expressed by $(\mu_i - \mu_j)/\bar{\mu}$. However, in order to illustrate the effect of the solvent for the different

anions, the selectivity coefficient as defined in Eq. (7) is taken as the base for discussion.

The different behavior of the two groups of anions as observed for the Walden product discussed above is also clearly seen here: the non-OH-substituted anions do not exhibit an overall change in the selectivity coefficients (Fig. 6a). Besides individual changes of some coefficients, the range of the coefficients is found between about 1 and 1.2, independently of the solvent composition; at 60% EtOH even a small reduction of the range is found. In contrast, the OH-substituted anions show an increase in this coefficient range with increasing ethanol concentration (Fig. 6b). Whereas the range is also between about 1 and 1.2 at 0% EtOH (salicylic acid, the solute with the highest mobility in all solvent systems, is the only exception), it increases

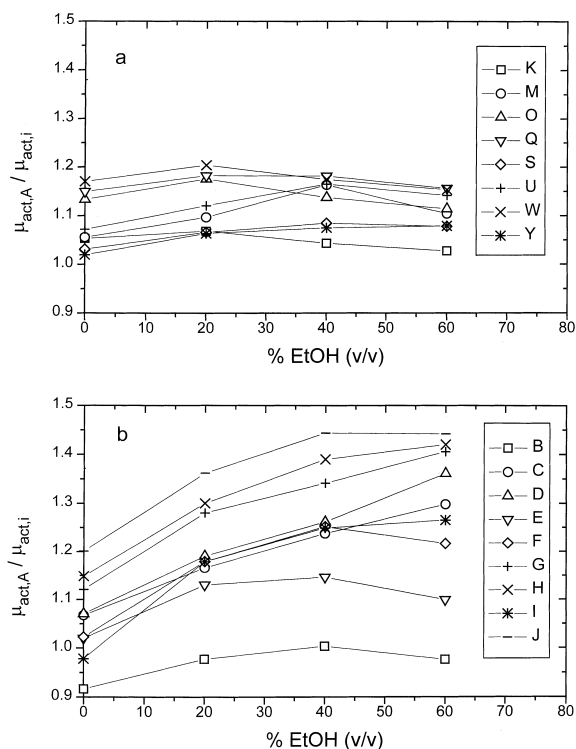


Fig. 6. Selectivity coefficients as function of the solvent composition for (a) the non-hydroxy-substituted and (b) the hydroxy-substituted anions. The symbols of the anions are according to Table 3. The selectivity coefficient is expressed by the ratio of the actual mobility of benzoate (A) to that of the solute, i , according to Eq. (7).

constantly up to 1.45 at 60% EtOH. It should be pointed out that this general trend is exactly that observed for the mixed aqueous–methanolic solvents. A discussion about individual selectivity effects considering a particular pair of solutes is, however, not the topic of the present investigation.

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